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LOWER MARINE FUNGUS ASSOCIATED WITH BLACK LINE DISEASE IN STAR CORALS (*MONTASTREA ANNULARIS,* E. & S.)

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Abstract

A disease of corals called "black line" has become widespread in the Caribbean reefs. Although its etiology has not been determined, a lower marine fungus was found closely associated with the disease. Corals of the species *Montastrea annularis* (star coral) were collected from scattered areas of the Venezuelan reefs. Histological examinations of black line-diseased corals showed this unidentified fungus in and nearby all of the diseased tissue. The branched fungal hyphae lacked septa and ranged in size from 5 to 10 μm long and from 2.5 to 3.0 μm wide. No hyphae were found in black line disease-free areas. No fungi have been detected previously in soft coral tissue. The study of this naturally occurring infection could yield important information concerning pathological processes in corals.

Introduction

Diseases in marine animals appear to be a common feature in the aquatic environment (Kinne, 1980). However, disease processes in marine animals have been rarely studied as biological phenomena. Not much is known about pathological conditions in cnidarians, especially in "true" corals (Anthozoa: Scleractinia). In 1975, Garrett and Ducklow first reported a naturally occurring disease in the scleractinian corals of the Bermudian reefs. Personal observations of similar conditions in the Venezuelan reefs prompted my study four years ago.

Black line-diseased corals have been found in several Atlantic reefs (Fig. 1): Bermuda (Garrett and Ducklow, 1975); Barbados (Ducklow, 1977); Florida (Voss, 1973 and pers. comm. from W. Jaap, Florida Department of Natural Resources, Marine Research Laboratory, 100 Eighth Avenue, S.E., St. Petersburg, FL 33701); Saint Thomas (Coki Bay) and Saint Croix (East Point and Buck Island), U. S. Virgin Islands (pers. ob., 1976); and Venezuela. No reports of this disease have been published concerning Pacific reefs.

Materials and Methods

A comparison of diseased tissue with normal tissue was made (Fig. 2). For this purpose, small coral heads of the species *Montastrea annularis* (Ellis and Solander), measuring about 3 cm in diameter, were collected from the Venezuelan reefs of Morrocoy National Park (10° northern latitude, 68° western longitude) and Los Roques National Park (11° northern latitude, 66° western longitude). These collection sites were chosen on the basis of field observations.

Three major collection sites were established for the study: 1) an area with a high occurrence of the disease (southern reefs of Cayo Norte, Morrocoy Park); 2)

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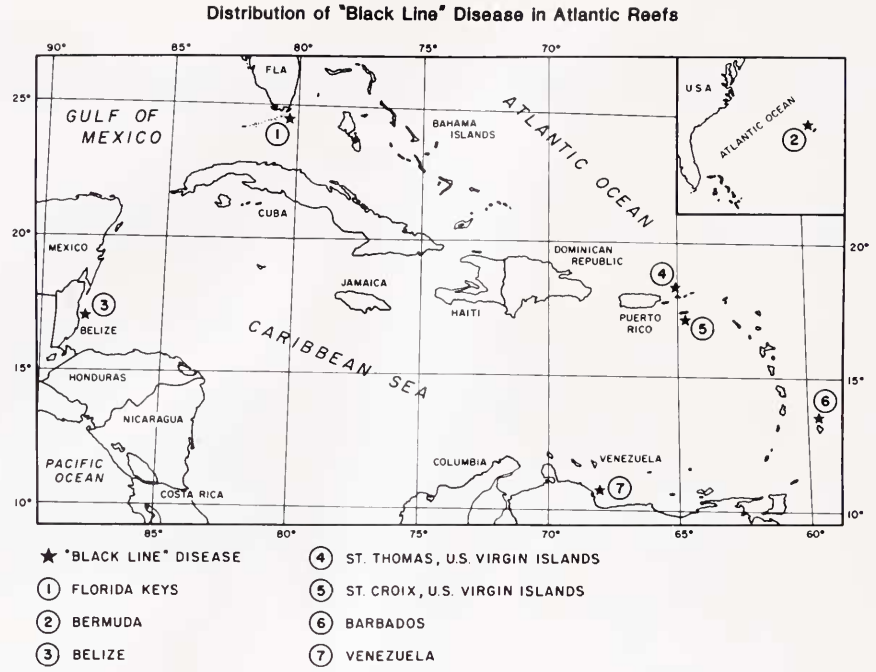

FIGURE 1. Distribution of "black line" disease in Atlantic reefs.

areas with moderate occurrences of the disease, ranging from 5 to 200 meters from the disease area (reefs west and northwest of Cayo Norte, Morrocoy Park); and 3) areas free of observable disease, ranging from 8 to 200 kilometers away from the

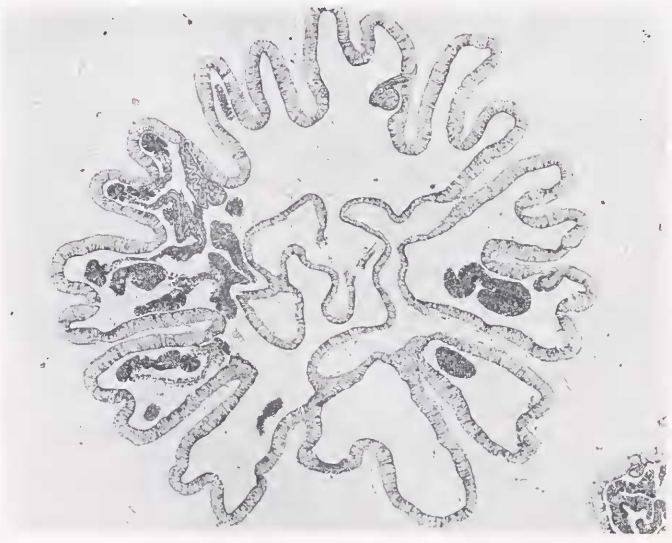

FIGURE 2. Cross section of a healthy *Montastrea annularis,* E. & S., polyp. Epidermis is free of invading organisms. Stained with toluidine blue O, methylene blue and borax. 40×.

diseased area (reefs of Cayo Sombrero in Morrocoy Park and reefs of Cayo Mosquito in Los Roques Park).

Coral heads were collected and fixed in solution for twenty-four hours. The fixative used was a modification of the formula given by McDowell and Trump (1977). The ingredients used were: 2 ml of 50% glutaraldehyde; 10 ml of 40% formaldehyde; 50 ml of filtered sea water; and 39 ml of filtered tap water. Ambient filtered sea water was used instead of the recommended buffer (sodium phosphate monobasic). The pH was adjusted to 7.4 with NaOH. The tissues were stored in alcohol until processed.

Small pieces of tissue were decalcified in Von Eber's decal (50 ml of 36% NaCl; 42 ml of distilled water and 8 ml of concentrated HCl). Small coral pieces took three days to decalcify, larger pieces took up to seven days and the decalcifying baths were changed daily. After decalcification, the tissues were washed, dehydrated in graded alcohols, and embedded in JB-4 (Polysciences), a glycol methacrylate polymer. Sections cut 1.5 microns thick were stained with toluidine blue O, methylene blue and borax dissolved in distilled water. The solution was prepared by adding 250 mg of toluidine blue O, 250 mg of methylene blue and 250 mg of borax to 100 ml of distilled water.

Other histological stains (Periodic acid Schiff, Giemsa, alcian blue and PAS at pH 1.0 and pH 2.5) were used also, as well as Grocott's method for fungi (GMS). The procedures for these stains are described in Luna (1968). After staining, the sections were mounted on plastic-coated slides, covered with a mounting medium and a cover slip, and examined under the microscope.

Results

Identification of the disease in the field

The gross appearance of the disease in the field is a dark (black) line separating the dead from the living tissue in a coral head (Fig. 3). The upper coral skeleton

FIGURE 3. Gross appearance of star coral (*Montastrea annularis*, E. & S.) presenting "black line" disease. 4×. (Picture was taken under the laboratory dissecting microscope.)

remains mostly intact until it is overgrown by algae and other organisms. No living tissue is observable within the circumference of the black line. Well beyond the black ring, the coral appears healthy and maintains all of its zooxanthellae. Discolored patches on the coral heads are often seen in affected areas. This discoloration may indicate an early stage of the disease and may result from the loss of zooxanthellae. The most commonly affected coral genera are *Diploria* (brain coral) and *Montastrea* (star coral).

For practical purposes, a healthy coral head and polyp were defined as being free of visible lesions. Moderately affected heads and polyps showed few fungi and/or filamentous algae near the affected tissue. Infection did not occur in all cases. Heavily affected heads and polyps showed massive fungal infection and the coral tissue was destroyed for the most part. Algal invasions were present in some cases.

Histological examination in the laboratory

One hundred and fifty-nine polyps from twelve different coral heads were examined histologically (Table I). The epidermis of all individual polyps presenting the disease was penetrated by fungal hyphae (Figs. 4, 5) and in more advanced stages of the disease the gastrodermis and mesoglea also were invaded. Within a single coral head, those polyps situated directly below the black line were most affected. Polyps 1 cm away from the disease ring showed less fungal invasion and polyps 5 cm away from the diseased ring had almost no invading hyphae. The tissue appeared to be normal in these areas.

Histologic examination of the black line area in *Montastrea annularis* revealed an ellipsoidal tangle of densely packed, parallel hyphae, filamentous cyanobacteria, algal fruiting bodies, diatoms, released zooxanthellae, and rodophytes. In some instances, there were mixed fungal and algal invasions of the polyp epidermis. However, although algae were present in both disease and disease-free areas, fungal hyphae were found only in areas where the black line disease occurred. The fungal hyphae were branched and non-septate, 5 to 10 μm in length and 2.5 to 3.0 μm in width. The branching fungal filaments were stained orthochromatically with toluidine blue O and were positive for the PAS and for the GMS tests for fungal identification.

Since no sexual or asexual fruiting bodies were present, the fungus cannot be identified at this time. However, Dr. Jan Kohlmeyer (Professor, University of North Carolina, Institute of Marine Sciences, Morehead City, NC 28557) and Dr. Charles E. Bland (Professor, Department of Botany, University of North Carolina, Chapel

TABLE I

Occurrence of "black line" disease in geographically separated Venezuelan reefs: relationship to the presence of the fungus

Gross appearance of coral heads	Distance from area affected by "black line" disease	Coral heads	Coral polyps
		# diseased/ # examined	# infected with fungi/# examined
healthy	8 km; 200 km*	0/4	0/54
moderately affected	5 m; 200 m*	1/4	6/30
heavily affected	0 meters*	4/4	75/75

* See text for exact location.

FIGURE 4. Hyphae infecting coral epidermis. Moderate infection PAS. 200×. Arrow points to infection site.

Hill, NC 27514) commenting on sample slides, believed the fungus probably belonged to the *lower* marine fungi.

DISCUSSION

Although fungi are very abundant in the marine environment (Kohlmeyer and Kohlmeyer, 1979) and appear to be major pathogens in some higher aquatic in-

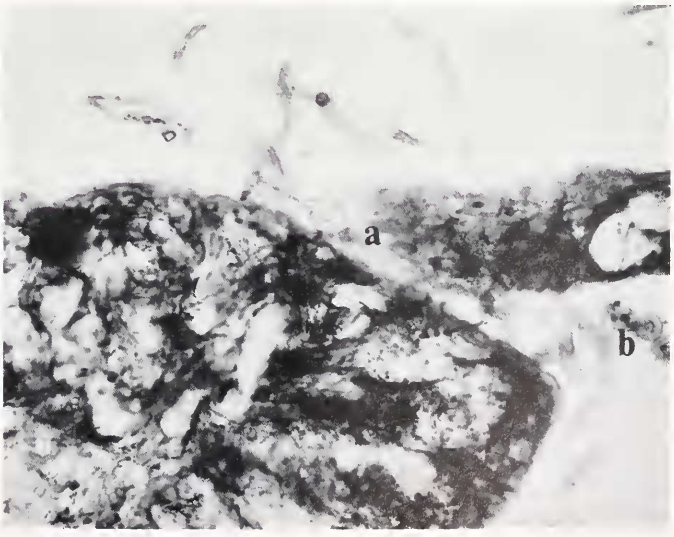

FIGURE 5. Closer view of infecting hyphae: a) hypha proliferation in the coral epithelium, and b) misplaced zooxanthellae PAS. 400×.

vertebrates such as crayfish and crabs (Nyhlèn and Unestam, 1975; Sparks and Hibbits, 1975), very little is known about their pathogenicity in lower aquatic invertebrates. In this study, histologic examination of black line disease in corals has shown that an invasion by fungal hyphae is associated with obvious pathological changes in the tissues. The possibility that this fungus may be a boring species is indicated by the presence of hyphae growing throughout the hard parts of the corals and within the septal invaginations. It is not possible at this time to determine whether the fungus is a primary or a secondary pathogen.

Other investigators have hypothesized that this disease may be caused by bacteria. Garrett and Ducklow (1975) have suggested a gram-negative filamentous *Beggiatoa* and a sulfate-reducing anaerobic *Desulfovibrio* as plausible pathogens. Antonius (1977) has suggested a filamentous cyanophyte, *Oscillatoria submembranacea* (Ardissone and Strafforelo) as the causative agent of the same coral condition. Nevertheless, no one has isolated the pathogen or reproduced the black line disease under controlled conditions.

The regenerative ability of some polyps may be a protective mechanism which prevents complete elimination of the reef. Nearly a century ago, Metchnikoff (1892) remarked on the amazing regenerative powers of coelenterates. The susceptibility of regenerating polyps to the disease is unknown, but some mechanism of differential susceptibility is likely since the disease does not always pursue a destructive course. Knowledge of individual polyp susceptibility to black line disease could lead to a determination of how a coral reef copes with advancing pathogens.

A large number of coral colonies on the reefs of Bermuda, Venezuela, and other Caribbean areas have dead patches. Since many of these patches may be disease related, the black line phenomenon may be an important factor in coral ecology. Knowledge of the etiology and pathogenesis of black line disease could, therefore, yield important clues to the manner in which corals defend themselves against parasites and other pathogenic agents.

Acknowledgments

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LITERATURE CITED

Antonius, A. 1977. Coral mortality in reefs: a problem for science and management. *Proc. Third Int. Coral Reef Symp.*, Miami, **2**: 617–620.

Ducklow, H. W. 1977. *Influence of Sublethal Pollutant Concentrations on the Microbial Ecology of Living Corals.* Ph.D. Dissertation, Harvard University.

GARRETT, P., AND H. W. DUCKLOW. 1975. Coral diseases in Bermuda. *Nature* **253**: 349–350.
KINNE, O. 1980. *Diseases of Marine Animals. I. General Aspects, Protozoa to Gastropoda.* John Wiley, Somerset, NJ. 460 pp.
KOHLMEYER, J., AND E. KOHLMEYER. 1979. *Marine Mycology: The Higher Fungi.* Academic Press, New York. 690 pp.
LUNA, L. G. 1968. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology.* Third ed. McGraw-Hill, New York. 258 pp.
MCDOWELL, E., AND B. F. TRUMP. 1977. Practical fixation techniques for light and electron microscopy. *Comp. Pathol. Bull.* **IX**: 3.
METCHNIKOFF, E. 1892. *Lectures on the comparative pathology of inflammation.* Translated from French by F. A. Starling and E. H. Starling. Dover Publications, New York, 1968. 224 pp.
NYHLÈN, L., AND T. UNESTAM. 1975. Ultrastructure of the penetration of the crayfish integument by fungal parasite, *Aphanomyces astaci,* Oomycetes. *J. Invert. Pathol.* **26**: 353–366.
SPARKS, A. K., AND J. HIBBITS. 1979. Black mat syndrome: an invasive mycotic disease of the tanner crab, *Chioecetes bairdi. J. Invert. Pathol.* **34**: 184–191.
VOSS, G. 1973. Sickness and death within the Florida reefs. *Nat. Hist.* **82**: 41–47.